

## Appearance of Immunoglobulin G Fc Receptor in Cultured Human Cells Infected with Varicella-Zoster Virus

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After infection with varicella-zoster virus, HeLa and human embryo lung cells developed a receptor for the Fc portion of human and rabbit immunoglobulin G. The receptor was detected by both hemadsorption and immunofluorescence, using antibody-coated erythrocytes and heat-aggregated immunoglobulin G. However, sheep erythrocytes sensitized with F(ab')<sub>2</sub> of anti-sheep erythrocyte antibody did not adsorb to the receptor. When cell-free varicella-zoster virus was inoculated into the HeLa cell monolayer, the Fc receptor appeared at first 6 h after infection; varicella-zoster virus antigen in the cytoplasm and detectable cytopathic effects appeared later.

Cells infected with herpes simplex virus developed the ability to bind sheep erythrocytes (SE) sensitized with rabbit anti-SE serum (11). The failure of the F(ab')<sub>2</sub> fragment of immune gamma globulin against SE to mediate the reaction had led to the suggestion that the phenomenon was due to the appearance of a receptor for the Fc fragment of immunoglobulin in the cell membrane (14, 15).

Recently, many believe that in addition to cells infected with herpes simplex virus cells infected with cytomegalovirus (4, 5, 9, 13) and Epstein-Barr virus (6) also exhibit an immunoglobulin G (IgG) receptor in their surface membranes. In the case of varicella-zoster virus (VZV), one of the human herpesviruses, hardly any reports have been made on the appearance of the Fc receptor on the membrane of infected cells. The present report describes aspects of an IgG receptor which appeared in cultured human cells after infection with VZV.

HeLa cells and human embryo lung (HEL) cells were cultured in Eagle minimal essential medium supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml), and 15% heat-inactivated fetal bovine serum for HEL cells or 10% fetal bovine serum for HeLa cells. HeLa and HEL cells were seeded in a 16-mm well, multi-dish tray (Linbro Scientific Co. Inc.) containing a glass cover slip. The tray was incubated at 37°C in a CO<sub>2</sub> incubator for 2 to 3 days, and the monolayers obtained were used for the experiment. Three strains of VZV isolated from patients with herpes zoster were used. VZV strain Ohtomo had been passaged at least 100 times in HEL cells, and the other two strains, Hirai and Kanno, were freshly isolated from patients and

passed 3 to 10 times in HEL cells. These three strains of VZV were identified with a reference immune serum (purchased from Flow Laboratories) by the immunofluorescence (IF) technique.

VZV-infected HEL cells were prepared by trypsinization of a HEL monolayer which had been infected with VZV. The trypsinized cells were suspended in a growth medium for HEL containing 10% dimethyl sulfoxide and then stored at -80°C until use. Cell-free VZV was prepared following the procedure reported by Schmidt and Lennette (10).

Purified human and rabbit IgG's were obtained from pooled human sera from three children and from normal rabbit serum, using diethylaminoethyl-cellulose chromatography. These sera were confirmed for the absence of anti-VZV antibodies by indirect IF and neutralization tests (titers < 1:5). The obtained IgG was adjusted to 20 to 50 mg/ml in a concentration of protein in phosphate-buffered saline (pH 7.2) and heated at 63°C for 15 min to obtain aggregated IgG (agg-IgG). Rabbit anti-SE serum and anti-Rh<sub>0</sub> (D) gamma globulin (Rhobulin) were purchased commercially. Anti-VZV serum was obtained by hyperimmunization of rabbits with the Ohtomo strain. The F(ab')<sub>2</sub> fragment from IgG of rabbit anti-SE sera was prepared by pepsin digestion according to the procedure of Nisonoff et al. in principle (7). The digested IgG was fractionated by gel filtration on a Sephadex G-100 column, and the major protein peak containing F(ab')<sub>2</sub> was further fractionated by carboxymethylcellulose chromatography, using the gradient buffer system of Porter (8), and concentrated by ultrafiltration (Minicon B-15, Amicon Corp., Lexington

ton, Mass.) to 0.64 mg of protein per ml.

In most experiments, hemadsorption (HAD) was carried out by using SE sensitized with rabbit anti-SE antibody as indicator cells (15). One-tenth milliliter of a 1% suspension of sensitized SE was added to the VZV-infected monolayer cells on a cover slip. The monolayers were incubated at 37°C for 30 min, and then the cover slips were rinsed in Hanks balanced salt solution containing 0.1% gelatin (GH medium) for the removal of unadsorbed SE. The monolayers were examined for HAD under  $\times 100$  magnification in a microscope, and the HAD was graded from 1<sup>+</sup> to 3<sup>+</sup> according to the degree of adherence of SE to the monolayer. A similar technique was used for the experiments employing human erythrocytes sensitized with Rhobulin as indicator cells.

The first experiment was carried out to examine HAD with sensitized SE and human erythrocytes and to determine what fraction of immunoglobulin of anti-SE serum was effective for HAD after sensitization of SE. HeLa cell monolayers on cover slips were inoculated with 20 VZV (Ohtomo)-infected HEL cells per cover slip and were incubated at 37°C for 2 days. When one of the rabbit anti-SE sera, which had a hemagglutinating antibody titer of 1:1,024, was used for the sensitization of SE, the SE sensitized with 1 unit of this antibody adsorbed to the surface of the VZV-infected HeLa cells. Unsensitized SE did not adsorb to virus-infected HeLa cells at all, and uninfected cells did not show adsorption of sensitized SE.

When similar experiments were performed with human erythrocytes (Rh positive) and Rhobulin, VZV-infected HeLa cells also exhibited HAD with the human erythrocytes sensitized with a 1:200 dilution of Rhobulin, although direct hemagglutination was negative at a 1:50 dilution (Table 1).

When HAD was carried out with IgG and IgM fractions of the same anti-SE antiserum, SE sensitized with a 1:512 dilution of IgG exhibited HAD on the infected HeLa cells, although SE were not agglutinable to each other. In contrast, SE sensitized with the same dilution of IgM of the antiserum markedly agglutinated but did not adsorb to the infected HeLa cells at all. Subsequently, the F(ab')<sub>2</sub> fraction of IgG from the same anti-SE serum retained hemagglutinating activity at a dilution of 1:160 against SE; however, the indicator cells sensitized with this dilution did not adhere to the foci of infected HeLa cells.

The second experiments were performed to find out the difference in the appearance of the Fc receptor between diploid and tumor cell lines, i.e., HEL and HeLa cells. Two cell lines were

propagated in a monolayer on cover slips and were infected with three strains of VZV by the inoculation of 20 to 30 infected HEL cells onto the monolayer. The infected cultures were incubated at 37°C for 48 h and tested for HAD with sensitized SE. The apparent HAD was observed with three strains of VZV (Table 2). In all cases, including the three VZV strains and a herpes simplex virus strain, HAD was always stronger in HeLa than in HEL cell cultures.

In the third experiment the presence of Fc receptors in infected HeLa cells was also demonstrated by adsorption of agg-IgG to the cells. About 20 HEL cells infected with the Ohtomo strain of VZV were inoculated onto the HeLa cell monolayer on a cover slip. At 48 h postinfection, the monolayers were washed and human or rabbit agg-IgG was introduced onto them at

TABLE 1. HAD mediated by erythrocytes sensitized with rabbit and human antibody to VZV-infected HeLa cells

Erythrocytes	Antibody	Reciprocal of dilution	Hemagglutination	HAD to HeLa cells <sup>a</sup>	
				VZV infected	Uninfected
Sheep	Rabbit anti-SE	512	+	2+	—
		1,024	+	1+	—
		2,048	—	—	—
Human	None		—	—	—
	Human anti-Rh <sub>0</sub> (D)	50	— <sup>b</sup>	1+	—
		100	—	3+	—
		200	—	2+	—
	None		—	—	—

<sup>a</sup> Positive reactions were graded from 1+ to 3+: 1+, less than 10% of infected area; 2+, 10 to 50%; 3+, more than 50%. —, Negative reaction.

<sup>b</sup> Positive for hemagglutination with indirect (Coomb's) method.

TABLE 2. HAD by HEL and HeLa cells infected with three strains of VZV

Virus	Strain	HAD to infected culture cells	
		HEL	HeLa
VZV	Ohtomo	1+	2+
	Hirai	2+	2+
	Kanno	2+	3+
HSV <sup>b</sup> type 1	Miyama	2+	3+
None		—	—

<sup>a</sup> HAD using SE sensitized with rabbit anti-SE (IgG, 1:256). Reactions scored as given in footnote a, Table 1.

<sup>b</sup> HSV, Herpes simplex virus.

an appropriate dilution in GH medium. After incubation for 30 min at 37°C, the monolayers were washed with GH medium and stained with indirect IF, using rabbit anti-human IgG or goat anti-rabbit IgG conjugated with fluorescein isothiocyanate. The human agg-IgG adsorbed to the infected HeLa cells at a concentration of 0.05 mg/ml, and the rabbit agg-IgG adsorbed to them at a concentration of 1 ml/ml. Thus, human IgG was approximately 20 times stronger than rabbit IgG in affinity to the infected cells. Neither type of IgG adsorbed to uninfected HeLa cells. Fluorescence was observed on the cell surface in small, disseminated, punctated, or irregular, patchy spots. The pattern of fluorescence was distinctly different from that of surface VZV antigen, which showed diffuse or circumscribed fluorescence on the cellular membrane.

The reaction was also seen when the VZV-infected cells were fixed in acetone for 10 min at room temperature. Human and rabbit agg-IgG bound to the acetone-fixed, infected HeLa cells at concentrations of 0.5 and 10 mg/ml, respectively, which was 10 times the concentration of agg-IgG necessary for binding to the unfixed, infected HeLa cells.

The last experiments were performed to trace

the time course of appearance of the Fc receptor in infected HeLa cultures as compared with the formation of viral antigens. The HeLa cell cultures were inoculated with cell-free virus (Kanno strain of VZV) at an input multiplicity of 0.05 plaque-forming unit/cell. One-fifth milliliter each of the virus suspension in maintenance medium was added to the cover slips, and after 2 h of virus adsorption at room temperature the medium was removed and 1 ml of fresh maintenance medium was added to each well before the tray was incubated at 37°C. At intervals indicated in Fig. 1, three cover slips were withdrawn and examined for HAD, agg-IgG binding, and synthesis of viral antigen by IF. HAD and the binding of agg-IgG were tested in unfixed cells, and the viral antigens were examined in acetone-fixed cells. Each experiment was carried out using three cover slips, and the average number of foci on the cover slip, including HAD- and IF-positive cells, was counted. The foci of HAD- or IgG-bound cells appeared 6 to 8 h after infection and increased in number until 24 h later (Fig. 1). The appearance of HAD-positive cells and IgG-bound cells consistently preceded the appearance of VZV antigen-positive cells, which was first observed 12 h after infection of

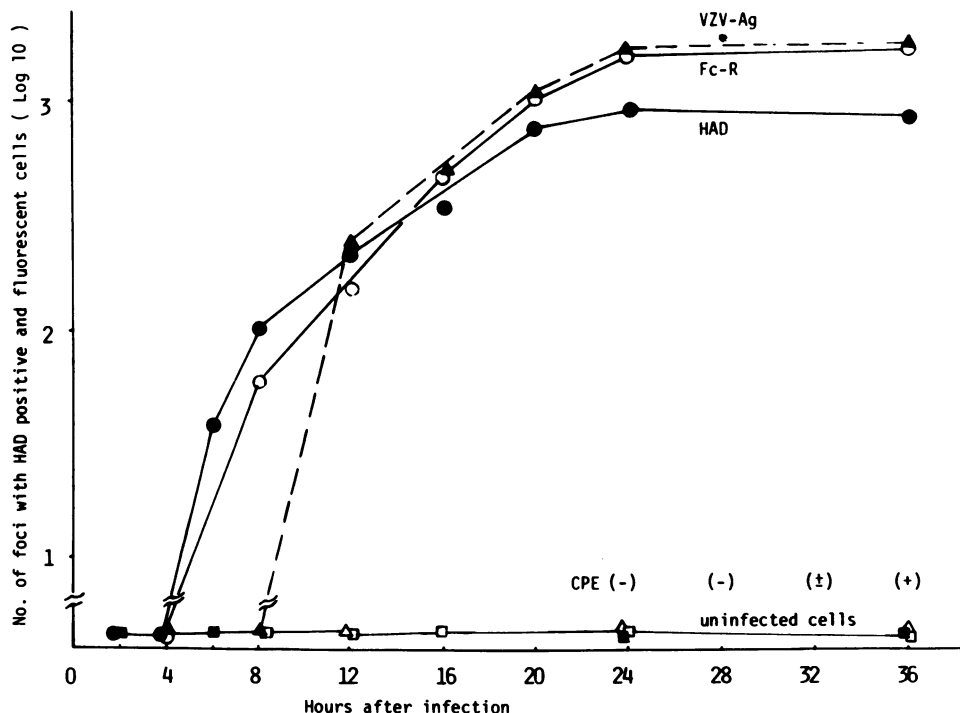


FIG. 1. Appearance of the Fc receptor and viral antigens in HeLa cells infected with cell-free VZV. Symbols: Number of foci with HAD-positive cells (●); Fc receptor-positive cells with binding of agg-IgG (○); viral antigen-positive cells (▲) in VZV-infected HeLa cell monolayer. Controls: foci with HAD (■), agg-IgG binding (□), and viral antigens (△) in an uninfected monolayer. CPE, Cytopathic effect.

the HeLa cell cultures. The number of foci with IgG-bound cells and that with VZV antigen-containing cells were almost the same after 20 h of infection. This result indicated that in the late stage of infection in the HeLa cells, most cells synthesizing VZV antigen were reactive to the agg-IgG. However, only 45% of the foci producing VZV antigen were detectable with HAD.

In this report, we confirmed that VZV-infected HeLa and HEL cells were adsorbed with rabbit or human IgG which had been coated on the erythrocytes or aggregated by heating. HAD was observed with three different strains of VZV and was always stronger in HeLa than in HEL cell cultures.

When the IgG fraction of rabbit anti-SE antibody was used for the sensitization of SE, HAD occurred in infected HeLa cells, but SE sensitized with the IgM fraction of anti-SE antibody did not adsorb. When the IgG of the antibody was treated with pepsin it lost the capacity to mediate HAD, although the capacity to agglutinate SE was retained. Thus, it was proved that HAD was performed by the Fc portion of the IgG antibody.

VZV-infected HeLa cells also exhibited HAD with human anti-Rh<sub>0</sub> (D) antibody. There seemed to be a difference in the affinity to human IgG between the HADs by VZV- and herpes simplex virus-infected cells. In our opinion, the Fc receptor induced by VZV is likely stronger in affinity to human IgG than in that to rabbit IgG, and this is quite contrary to the receptor induced by herpes simplex virus, which had shown stronger HAD with rabbit IgG than with human IgG (15). The presence of the Fc receptor in VZV-infected HeLa cells was also demonstrated by adsorption of heat-aggregated IgG on their surface. Both human and rabbit agg-IgG, in which no detectable antibody was present, had adsorbed to the surface of VZV-infected HeLa cells, and the IgG was detected by indirect IF. It is well known that Fc receptor induced by cytomegalovirus has been detected in acetone-fixed cells by IF staining (4, 5) and binding of <sup>125</sup>I-labeled IgG (13). Furukawa et al. reported that normal human sera, which were negative in complement-fixing antibody to cytomegalovirus, bound to the cytomegalovirus-infected cell surface at a high dilution (4). We also found that human agg-IgG bound to the acetone-fixed VZV-infected cells, although the concentration of IgG needed was 10 times higher than that used for binding to unfixed, infected HeLa cells. However, in our experiences during the study of VZV, human IgG from anti-VZV antibody-negative serum did not adsorb to VZV-infected cells unless it was agg-IgG. During the titration of serum samples to detect antibody to

VZV by indirect IF, we found many antibody-negative samples (titers < 1:5) and never observed the nonspecific binding of immunoglobulin to unfixed or acetone-fixed, VZV-infected cells. It is conceivable that these findings are due to the biological difference in the Fc receptors induced by VZV and cytomegalovirus.

Comparing our findings with those of others, we suggest that the difference in the properties of Fc receptor induced by VZV and other herpesviruses is as follows. (i) VZV-infected cells bound human agg-IgG more strongly than rabbit agg-IgG and did not adsorb native human IgG. (ii) Cytomegalovirus-infected cells also bound human IgG more strongly than rabbit IgG (5, 13) but adsorbed native human IgG (4, 5). (iii) However, herpes simplex virus-infected cells bound both human and rabbit IgG's to a similar extent (3) or sometimes slightly more strongly in rabbit IgG than in human IgG (14), and they also adsorb native human IgG (3).

From the time course study of the infection of cell-free VZV in HeLa cells, the appearance of both HAD-positive and agg-IgG-bound cells consistently preceded the appearance of VZV antigen-positive cells. Similar results were observed in investigations with herpes simplex virus-infected (1, 2, 12, 13) and cytomegalovirus-infected (4, 13) cells.

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#### LITERATURE CITED

- Adler, R., J. C. Glorioso, J. Cossman, and M. Levine. 1978. Possible role of Fc receptors on cells infected and transformed by herpesvirus: escape from immune cytolysis. *Infect. Immun.* 21:442-447.
- Costa, J., C. Yee, Y. Nakamura, and A. Rabson. 1978. Characteristics of the Fc receptor induced by herpes simplex virus. *Intervirology* 10:32-39.
- Feorino, P. M., S. L. Shore, and C. B. Reimer. 1977. Detection by indirect immunofluorescence of Fc receptors in cells acutely infected with herpes simplex virus. *Int. Arch. Allergy Appl. Immunol.* 53:222-233.
- Furukawa, T., E. Hornberger, S. Sakuma, and S. A. Plotkin. 1975. Demonstration of immunoglobulin G receptors induced by human cytomegalovirus. *J. Clin. Microbiol.* 2:332-336.
- Keller, R., R. Peitchel, J. H. Goldman, and M. Goldman. 1976. An IgG-Fc receptor induced in cytomegalovirus-infected human fibroblasts. *J. Immunol.* 116:772-777.
- Nishioka, K., T. Tachibana, T. Hirayama, G. de Thé, G. Klein, M. Takada, and A. Kawamura, Jr. 1971. Immunological studies on the cell membrane receptors of cultured cells derived from nasopharyngeal cancer, Burkitt's lymphoma, and infectious mononucleosis, p. 401-420. *In* W. Nakahara, K. Nishioka, T. Hirayama, and Y. Ito (ed.), *Recent advances in human tumor virology and immunology*. Japan Scientific Societies

- Press, Tokyo.
7. Nisonoff, A., F. C. Wessler, and L. N. Lipman. 1960. Properties of the major component of a peptic digest of rabbit antibody. *Science* **132**:1770-1771.
  8. Porter, R. R. 1959. The hydrolysis of rabbit  $\gamma$ -globulin and antibodies with crystalline papain. *Biochem. J.* **73**: 119-126.
  9. Rahman, A. A., M. Teschner, K. K. Sethi, and H. Brandis. 1976. Appearance of IgG (Fc) receptor(s) on cultured human fibroblasts infected with human cytomegalovirus. *J. Immunol.* **117**:253-258.
  10. Schmidt, N. J., and E. H. Lennette. 1976. Improved yields of cell-free varicella-zoster virus. *Infect. Immun.* **14**:709-715.
  11. Watkins, J. F. 1964. Adsorption of sensitized sheep erythrocytes to HeLa cells infected with herpes simplex virus. *Nature (London)* **202**:1364-1365.
  12. Watkins, J. F. 1965. The relationship of the herpes simplex haemadsorption phenomenon to the virus growth cycle. *Virology* **26**:746-753.
  13. Westmoreland, D., S. S. Jeor, and F. Rapp. 1976. The development by cytomegalovirus-infected cells of binding affinity for normal human immunoglobulin. *J. Immunol.* **116**:1566-1570.
  14. Westmoreland, D., and J. F. Watkins. 1974. The IgG receptor induced by herpes simplex virus: studies using radioiodinated IgG. *J. Gen. Virol.* **24**:167-178.
  15. Yasuda, J., and F. Milgrom. 1968. Hemadsorption by herpes simplex-infected cell cultures. *Int. Arch. Allergy* **33**:151-170.